

### **Remarks**

Claims 1-3 and 7 were pending prior to this Response. Applicants note that claims 6 and 8-15 were canceled in the Preliminary Amendment filed January 23, 2006. Thus, reference to claims 1-3 and 6-15 as being pending on page 4 of the Response filed August 16, 2006, was a typographical error.

By the present communication, no claims have been added or canceled, and claims 1-3 and 7 have been amended to define Applicants' invention with greater particularity. The amendments do not raise any issues of new matter and the amended claims do not present new issues requiring further consideration or search. Support for amended claim 2 may be found, among others, at page 10, lines 1-3. Accordingly, claims 1-3 and 7 are currently pending in this application.

### **Claim Objections**

Applicants respectfully traverse the objection to claim 2 as allegedly failing to further limit the subject matter of a previous claim. Applicants have amended claim 2 to set forth the retention of serine, glutamic acid and lysine residues. Accordingly, Applicants respectfully request withdrawal of the objection.

### **Rejection under 35 U.S.C. § 112, Second Paragraph**

Applicants respectfully traverse the rejection of claims 1-3 and 7 as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner alleges that in claim 1 it is unclear what is intended by the recitation, "...except that and lysine residues of the natural toxin in its amino acid sequence, except that a formalin...." Applicants have amended claim 1 to correct the apparent typographical error by removing the first "except that" from the recitation. Withdrawal of the rejection is respectfully requested.

Further, the Examiner alleges that claims 2 and 3 depend on claim 1, but claim 1 refers to two different toxins. In order to reduce the issues and further prosecution, Applicants have

amended claims 2 and 3 to clarify which toxin is being further limited. Withdrawal of the rejection is respectfully requested.

Finally, the Examiner alleges that claim 7 recites a dependency to a canceled claim. In order to reduce the issues and further prosecution, Applicants have amended claim 7 to correct the claim dependency. Withdrawal of the rejection is respectfully requested.

**Rejection under 35 U.S.C. § 102**

Applicants respectfully traverse the rejection of claims 1, 3, 6, 7 and 13 under 35 U.S.C. §102(b) as allegedly anticipated by Germanier, et al (hereinafter "Germanier"). Specifically, the Examiner alleges that Germanier discloses a purified and attenuated cholera toxin, wherein the toxin has a residual toxic activity of less than 1/2000 that of the natural toxin. Applicants submit that Germanier discloses cholera toxin (CT) as a starting material, which is first converted into procholeraegenoid by heat treatment, and then the procholeraegenoid is treated with formalin to obtain an attenuated toxin. In contrast, the attenuated toxin of the present invention is produced *without* the heat treatment step. In other words, the claimed attenuated toxin is produced by directly treating CT with formalin, rather than treating procholeraegenoid with formalin.

Applicants further submit that the difference in production methods results in structural differences of the resulting attenuated toxins. The structural differences between CT and procholeraegenoid are shown in the table below. CT contains many cysteine residues, which form stable disulfide bonds (SS bonds) in a native environment (Lai, CY *et al.*, Biochem Biophys Res Commun. 116:341-8, 1983) (Abstract attached as Exhibit 1). When CT enters the body, the -SH group of the cysteine residues is released, thereby allowing the cysteine residues to form new SS bonds, which exerts toxin activity. Due to the SS bonds within the molecule, heat-treated CT undergoes structural and functional changes, resulting in procholeraegenoid, which has entirely different characteristics. Attached as Exhibit 2, Pierce, *et al.* describe "procholeraegenoid" as a stable, high molecular weight aggregate containing both the A and B subunits (see p. 1112, right column, Pierce, *et al.*, Infection and Immunity, 40(3):1112-8, 1983). Since the structural differences between CT and procholeraegenoid is evident, Applicants submit that one of skill in

the art would unambiguously distinguish the adjuvant of the present invention (comprising an attenuated CT) from the attenuated procholeraenoid of Germanier.

|  | Untreated CT  | Heat-treated CT<br>(i.e., procholeraenoid)                |
|--|---|---|
| <b>Solubility in water</b>                         | Soluble   | Insoluble (syrup or rubber-like form)                     |
| <b>Protein Denaturation</b>                        | Not denatured   | Denatured   |
| <b>Molecular weight</b>                            | Constant at 85 kD   | Multimeric complex at 85 kD or greater                    |
| <b>Amino Acid Sequence<br/>(primary structure)</b> | Constant  | Not constant (may have many changes in primary structure) |
| <b>SS Bond in the Amino Acid<br/>Sequence</b>      | Positions and the number of<br>SS bonds are constant )six<br>bonds between the A1 and A2<br>subunits and within the B<br>subunit) | Positions of SS bonds are not<br>constant or not clear    |
| <b>Protein Tertiary Structure</b>                  | Constant  | Not constant  |
| <b>Toxin Activity</b>                              | Active  | Less active than the untreated<br>CT                      |

Further, Applicants submit that Germanier is absolutely silent with regard to the adjuvant activity of the attenuated procholeraenoid. Germanier discloses that the procholeraenoid obtained by heat and formalin treatment is "antigenic" (see page 1696). However, Germanier does not disclose that the procholeraenoid retains an activity to enhance production of an antibody specific to an antigen other than the procholeraenoid itself (i.e., adjuvant activity). The only disclosure upon which Germanier may rely that relates to antibody production against antigens other than procholeraenoid is, "After immunizing rabbits with 100 µg of formalinized procholeraenoid, a weak but distinct rise in vibriocidal antibodies was observed." (p. 1696, right column, first full paragraph). However, in the same paragraph, Germanier concludes that residual somatic antigens present in the toxin preparation S-2, which was used to produce the procholeraenoid, would be the cause of the vibriocidal antibody production. Germanier suggests using the purer toxin preparation A-2 to prepare a toxoid free of somatic antigens (p. 1697, last paragraph). Accordingly, one of skill in the art may, at most, recognize that (i) some

somatic antigens inactivated neither by heat nor by formalin treatment may exist in Germanier's purified toxin preparation, and (ii) to eliminate such contaminating somatic antigens, a toxin preparation that has been highly purified through gel filtration or such techniques should be used to produce an attenuated procholera toxin. However, Applicants submit that one of skill in the art cannot reach the conclusion that Germanier's attenuated procholera toxin retains an adjuvant activity.

Anticipation under 35 U.S.C. § 102(b) requires that the reference recite each and every element of the claims in a single document. Since Germanier fails to disclose each and every element of the invention adjuvant, as defined by amended claim 1, Applicants respectfully submit that the Examiner has failed to establish anticipation under 35 U.S.C. § 102 (b) over Germanier, et al. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

### **Rejection under 35 U.S.C. § 103**

Applicants respectfully traverse the rejection of claim 7 under 35 U.S.C. § 103(a) as allegedly unpatentable over Germanier. The burden of proof in establishing a *prima facie* case of obviousness under § 103 clearly rests with the Patent Office. *In re Piasecki*, 745 F.2d 1468, 1472 (Fed. Cir. 1984). In establishing a *prima facie* case, the Patent Office, among other things, must show that (1) the prior art would have suggested to those of ordinary skill in the art that they should make the claimed invention and (2) that the prior art would have revealed a reasonable expectation of success. *In re Vaeck*, 947 F.2d 488, 493 (Fed. Cir. 1991). "Both the suggestion and the reasonable expectation of success must be found in the prior art, not in the applicant's disclosure." *Id.* Thus, "particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed." *In re Kotzab*, 217 F.3d 1365, 1371 (Fed. Cir. 2000). Further, when relying on the knowledge of persons of ordinary skill in the art, the Patent Office must "explain what specific understanding or technological principle within the knowledge of one of ordinary skill in the art would have suggested the combination." *In re Rouffet*, 149 F.3d

1350, 1357 (Fed. Cir. 1998). “The factual inquiry whether to combine references must be thorough and searching. It must be based on objective evidence of record. This precedent has been reinforced in myriad decisions, and cannot be dispensed with.” *In re Sang Su Lee*, 277 F.3d 1338, 1343 (Fed. Cir. 2002) (citations omitted).

Specifically, the Examiner alleges that it would have been obvious to one of skill in the art to remove all toxicity from the toxin to ensure its safe use with whole cell vaccines against *Vibrio cholerae* infection. Applicants cannot find disclosure of Germanier for “the administration of the detoxified toxin with whole cell vaccines against *Vibrio cholerae* infection.” As such, Applicants invite the Examiner to identify the specific passage within Germanier that discloses administration against *Vibrio cholerae* infection.

As discussed above, the structure of the claimed attenuated CT is different from that of the attenuated procholeraenoid of Germanier. The present invention teaches that a high degree of detoxification can be accomplished by simply formalin-treating a toxin that retains serine residues, glutamic acid residues, and lysine residues of the natural toxin. This detoxification is accomplished without treatment with heat. Further, Germanier is absolutely silent with regard to the retention of an adjuvant activity by the resulting procholeraenoid.

Accordingly, Applicants submit that Germanier does not suggest that use of CT, rather than procholeraenoid, would result in an attenuated toxin. Withdrawal of the rejection is respectfully requested.

In re Application of:  
Aizawa et al.  
Serial No.: 09/830,019  
Filed: September 21, 2001  
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**Conclusion**

In summary, for the reasons set forth herein, Applicants maintain that claims 1-3 and 7 clearly and patentably define the invention and respectfully request that the Examiner withdraw all rejections and pass the application to allowance. If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

No fee is believed due in connection with the filing of this Response. However, if any fee is due, the Commissioner is hereby authorized to charge any additional amounts required by this filing, or credit any overpayment, to Deposit Account No. 07-1896 referencing the above-identified attorney docket number. A duplicate copy of the Transmittal Sheet is enclosed.

Respectfully submitted,

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